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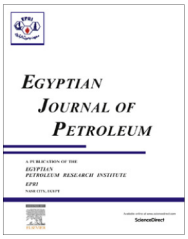

FULL LENGTH ARTICLE

Enzymatic biodiesel production from palm oil and palm kernel oil using free lipase

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Design Expert Software

Abstract Biodiesel from biological materials is receiving attention as alternative fuel. This investigation compared quality of biodiesel produced from lipase-transesterified palm oil (PO) and palm kernel oil (PKO) based on fatty acid methyl esters (FAME) and fuel properties. Biodiesel yield was optimized using three-level four-factor of Design Expert Software with enzyme load (2.5–7.5%), methanol-oil molar ratio (3-1, 1), and temperature (30–40 °C) as variables. Biodiesel properties FAME, Flash Point (FC), Pour Point (PP) and kinematic viscosity were compared with American (ASTM D6751) and European (EN 14214) Standards. PO (>90%) biodiesel yield was higher than PKO (<90%), both with maximum yields observed at 40 °C, 3:1 and 5–7.5%. FAME in PO-biodiesel (POBD) and PKO-biodiesel (PKOBD) include Hexadecanate and 9-Octadecenoate, while POBD had more unsaturated FAME (Dodecanoate). POBD and PKOBD had PP 6.7 °C and 17.7 °C respectively, while POBD Kinematic viscosity (813 kg/m^3) agreed with both standards. This study showed that POBD could be a better fuel alternative with further improvement of fuel properties.

1. Introduction

Energy is the most fundamental requirement for human existence and activities; however, non-renewable energy source that contributes over 86% of the global energy supply is depleting [1]. Shortage of resources and high crude oil prices have led to the search for a new alternative and renewable energy source such as biodiesel [2].

Biodiesel is renewable energy fuel consisting of long chain fatty acid derived from vegetable oils or animal fats and is non-toxic, biodegradable, renewable, does not contain sulfur and aromatic compounds, among other advantages [3,4]. Enzymatic conversion of oils to biodiesel by lipases as biocatalysts is receiving much interest in biodiesel production due to its high efficiency, selectivity and production of a highly purified product [5]. Oils from various feedstocks are used for the enzymatic production of biodiesel, with vegetable oil currently being used worldwide as a sustainable commercial feedstock [3]. Investigations have also been carried out into the use of other edible oils and feedstocks such as sunflower, soyabeans,

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rapeseed, corn etc. to supplement competition for vegetable oil with food producers which results in concerns of food security.

Palm oil and palm kernel oil are found in the flesh and seed (kernel) of the palm fruit respectively. They are two of the major crop found in Nigeria, being a tropical country with a wide variation of climatic and soil condition [6]. A 2008 report showed that palm oil and palm kernel rank first and third in the estimated list of annual production of major Nigerian vegetable oils, while the production size is also expected to increase [7]. This projection indicates the possibility of continued use of these oils in biodiesel production. This research investigation aimed at producing biodiesel from palm and palm kernel oil using free lipase.

2. Materials and methods

Lipase-producing strain of *Aspergillus niger* was obtained from the Culture Collection Center of Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria. Palm oil (PO) and palm kernel oil (PKO) were obtained locally from an oil palm industry. All chemicals used including tween 80, gum arabic, thymolphtalein, sodium dihydrogen phosphate, monosodium hydrogen phosphate, sodium potassium tartarate, copper sulfate pentahydrate were of analar grade.

2.1. Lipase production and assay

Lipase production by *A. niger* via solid state fermentation was done as previously described [8], while lipase activity was determined using a combined method [9,10]. Spores of the mold was inoculated in medium containing rice bran, palm kernel cake, groundnut cake and starch (5:5:3:1 w/w), moistened with distilled water to 55% and incubated at 30 °C for 72 h. The fermented moldy bran was mixed with 50 mM sodium phosphate buffer pH 8 (1:10w/v) and placed in an orbital shaker at 150 rpm at 28 °C for 2 h. The mixture was filtered using glass microfiber and the clear filtrate was used as crude lipase.

Olive oil substrate emulsion was prepared by mixing 25 ml of olive oil with 75 ml of 7% Arabic gum solution in a conical flask and incubate at 37 °C for 15 minutes using a water bath (Nickel Electro Ltd, England). Reaction mixture was made up of 50 ml olive oil emulsion and 10 ml crude enzyme incubated at 50 °C for 30 min with intermittent shaking in water bath. At 5-min intervals, 5 ml of reaction mixture was removed and mixed with 5 ml ethanol (95%) and thymolphtalein indicator (2–3 drops) in a conical flask to stop the reaction. The released fatty acid was titrated with sodium hydroxide (0.05 N) in a buret until a light blue color appears. Control experiment was done by mixing phosphate-buffered olive oil (5 ml) with

Table 1 Palm oil (PO) biodiesel yield (%) of different experimental runs.

Run	Factor 1 A:Temp (Celsius)	Factor 2 B:molar ratio (Molarity)	Factor 3 C:enzyme load (percent)	Factor 4 D:agitation (rpm)	Response biodiesel yield (percent)
1	35.00	2.00	5.00	220.71	94
2	30.00	3.00	2.50	100.00	94
3	30.00	1.00	2.50	100.00	90
4	30.00	1.00	7.50	100.00	90
5	35.00	2.00	5.00	150.00	94
6	35.00	2.00	5.00	150.00	94
7	40.00	3.00	7.50	200.00	95
8	35.00	2.00	5.00	150.00	94
9	40.00	1.00	2.50	200.00	93
10	40.00	3.00	2.50	100.00	94
11	35.00	0.59	5.00	150.00	90
12	35.00	2.00	5.00	150.00	94
13	30.00	3.00	2.50	200.00	94
14	40.00	1.00	7.50	200.00	93
15	40.00	1.00	2.50	100.00	93
16	35.00	2.00	5.00	79.29	94
17	35.00	3.41	5.00	150.00	95
18	30.00	3.00	7.50	200.00	93
19	40.00	3.00	7.50	100.00	95
20	40.00	1.00	7.50	100.00	90
21	35.00	2.00	5.00	150.00	94
22	30.00	1.00	2.50	200.00	89
23	40.00	3.00	2.50	200.00	94
24	27.93	2.00	5.00	150.00	93
25	35.00	2.00	5.00	150.00	94
26	35.00	2.00	1.46	150.00	93
27	30.00	3.00	7.50	100.00	93
28	30.00	1.00	7.50	200.00	90
29	35.00	2.00	8.54	150.00	92
30	42.07	2.00	5.00	150.00	95

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Table 2 Palm kernel oil (PKO) biodiesel yield (%) of different experimental runs.

Run	Factor 1 A:Temp. (Celsius)	Factor 2 B:molar ratio (Molarity)	Factor 3 C:enzyme load (percent)	Factor 4 D:agitation (rpm)	Response1 biodiesel yield (percent)
1	40.00	1.00	2.50	200.00	81
2	35.00	3.41	5.00	150.00	86
3	35.00	2.00	5.00	150.00	85
4	27.93	2.00	5.00	150.00	83
5	35.00	2.00	5.00	150.00	85
6	35.00	2.00	5.00	150.00	85
7	40.00	1.00	2.50	100.00	81
8	30.00	3.00	7.50	200.00	84
9	42.07	2.00	5.00	150.00	85
10	40.00	3.00	7.50	200.00	86
11	30.00	1.00	2.50	100.00	81
12	35.00	2.00	5.00	220.71	83
13	30.00	3.00	2.50	200.00	85
14	30.00	1.00	7.50	100.00	81
15	30.00	3.00	2.50	100.00	85
16	30.00	1.00	2.50	200.00	81
17	30.00	3.00	7.50	100.00	84
18	35.00	2.00	5.00	150.00	85
19	35.00	2.00	5.00	150.00	85
20	40.00	1.00	7.50	200.00	83
21	40.00	3.00	2.50	200.00	86
22	35.00	2.00	5.00	79.29	85
23	40.00	1.00	7.50	100.00	83
24	35.00	0.59	5.00	150.00	80
25	30.00	1.00	7.50	200.00	79
26	40.00	3.00	7.50	100.00	86
27	40.00	3.00	2.50	100.00	85
28	35.00	2.00	5.00	150.00	85
29	35.00	2.00	8.54	150.00	84
30	35.00	2.00	1.46	150.00	82

gum arabic emulsion substrate mixture and titrating with sodium hydroxide (0.05 N).

The quantity of fatty acid liberated is equivalent to the volume of NaOH used and it was calculated using Eq. (1), where N is the normality of NaOH titrant used (0.05 in this case).

$$\mu\text{mole fatty acid per ml sample} = \frac{((\text{ml NaOH of sample} - \text{mlNa OH of blank}) * N * 1000)}{5} \quad (1)$$

One unit (U) of lipase activity is defined as the amount of enzyme that releases from the emulsion substrate 1 μmole of fatty acid per ml per minute under specific assay condition.

2.2. Enzymatic production of biodiesel (fatty acid methyl ester) by free lipase

Transesterification reaction for PO and PKO was carried out in a separate 250 ml conical flask, using a rotary orbital shaker accordingly [11]. Lipase (2.5%) was added to 40 g of PO and PKO respectively and the mixtures were supplemented with methanol (5 ml) in a molar ratio 1:1 to the oil. Reaction mixture was left for 48 hours after which it was separated overnight using a separating funnel. Following separation, two layers were formed: the upper layer being the biodiesel product while glycerin settled at the bottom layer of the separating funnel.

2.3. Biodiesel yield

The fatty acid methyl ester, FAME, yield or biodiesel yield (% wt), relative to the amount of experimental oil used was calculated by comparing the weight of the upper layer biodiesel to the weight of the crude oil used [12] as described in Eq. (2).

$$\text{Biodiesel yield } (\%) = \frac{\text{Weight of biodiesel produced}}{\text{Weight of crude oil used}} \times 100 \quad (2)$$

2.4. Optimization of biodiesel production from PO and PKO by free enzyme

Optimization of the transesterification process of PO and PKO into biodiesel was investigated using a three-level four-factor Response Surface Central Composite Design (RSCCD) of Design Expert Software version 7b1.1 [11]. Biodiesel production factors enzyme load (2.5–7.5%), methanol to oil molar ratio (1–3, with oil fixed at 1), agitation (100–200 rpm) and temperature of reaction (30–40 °C) were the independent variables. The study required 30 experimental runs for each oil sample and the actual level of the independent variables as designed in different combination of factors (Tables 1 and 2). The experiments were carried out in a randomized order for 48 h reaction time. The product of transesterification of

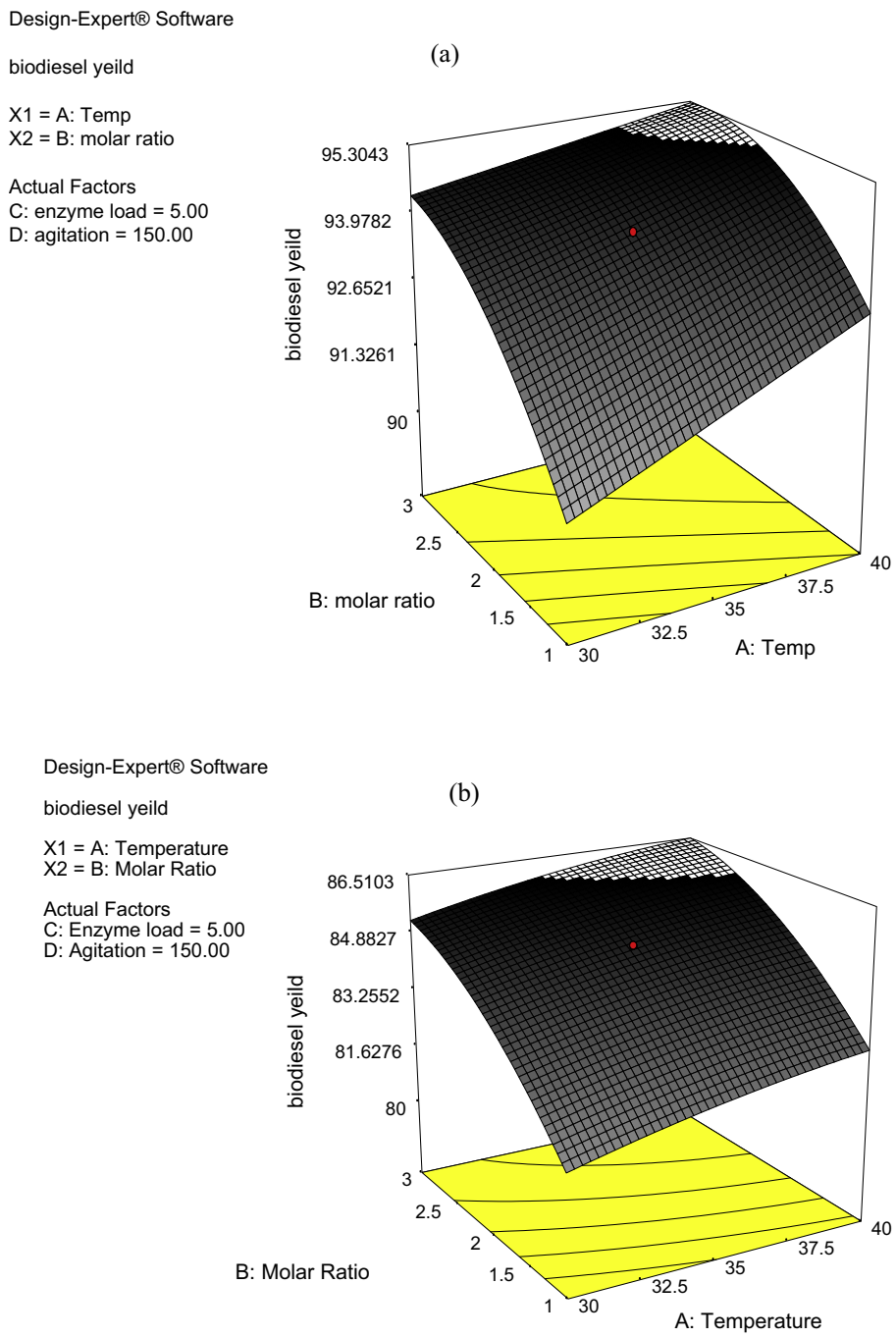

Figure 1 Effect of temperature and molar ratio on (a) PO and (b) PKO biodiesel yield.

each experimental run was allowed to settle for 24 h in a separating funnel and the fatty acid methyl ester (FAME) was separated from the glycerol layer as previously described.

2.5. Characterization of produced biodoesel

The fatty acid methylester (FAME) properties of biodiesel produced were analyzed using Gas Chromatography system (Agilent Technologies 7890A model) as previously described [13]. Properties such as Flash Point (FC), Cloud Point (CP), Pour Point (PP), kinematic viscosity and density at 15 °C of crude transesterified PO and PKO were carried out and compared with the American Standard of Testing material (ASTM 6751–3) and European Union Standard (EN 14214) in properties and quality of biodiesel. All properties were determined in triplicate.

3. Results and discussion

Response Surface Linear of Analysis of Variance (ANOVA) used in analyzing the response biodiesel yield of PO and PKO gave a model *F*-Value of 12.63 and 15.27 respectively

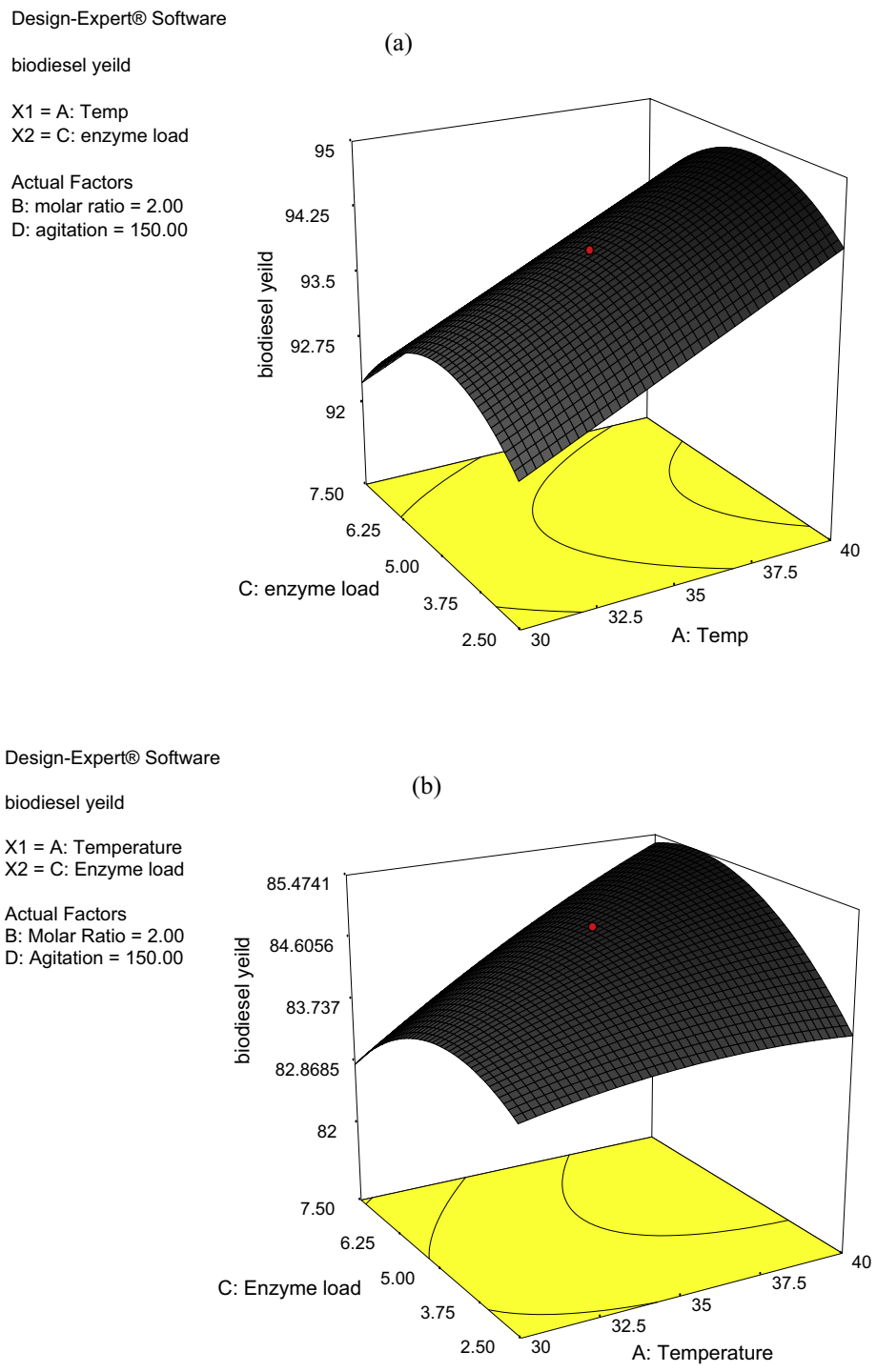

Figure 2 Effect of enzyme load and temperature on (a) PO and (b) PKO biodiesel yield.

and these demonstrate high significance of the model. The combined effect of temperature and molar ratio on PO and PKO biodiesel yield showed that maximum biodiesel yield of 95.30% and 86.55% was observed at a temperature of 40 °C and molar ratio of (3:1), while a minimum biodiesel yield of 89% and 79% respectively was observed at 30 °C and 1:1 molar ratio (Fig. 1a and b).

Similarly, effect of temperature and enzyme load on PO and PKO biodiesel yield was described in response surface plots in Fig. 2a and b respectively. It was observed that 7.5% enzyme load employed at and temperature of 40 °C were conditions required for optimum biodiesel yields of 95% and 86.94% from PO and PKO respectively. However, least biodiesel yield of 92% and 82.00% was observed at 30 °C and enzyme load of 2.50%.

Effect of molar yield and enzyme load on biodiesel yield from PO and PKO is described in Fig. 3a and b respectively. Maximum yield of 95% and 86.04% was recorded at molar

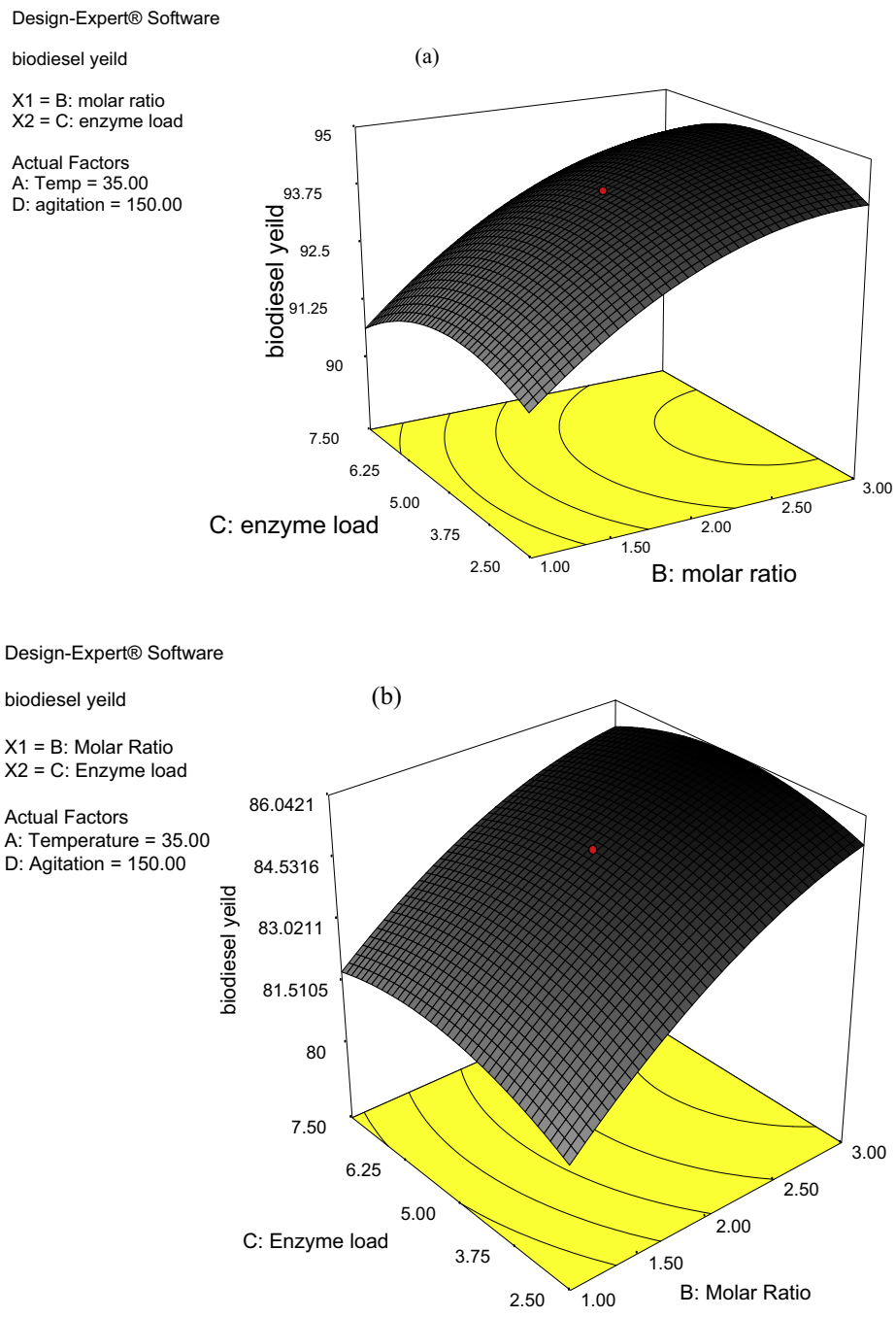

Figure 3 Effect of molar ratio and enzyme load on (a) PO and (b) PKO biodiesel yield.

ratio (3:1) and enzyme load of 5% for PO and PKO, while minimum yield of 90% and 82.5% was observed at molar ratio (1:1) and enzyme load 2.50% for both PO and PKO respectively. It was also observed that minimum biodiesel yield from PO was higher than maximum obtainable from PKO at the same conditions. Summary of the plots describe highest biodiesel yield of 95% achieved at temperature 40 °C, 3:1 molar ratio and 5% enzyme load. This is in agreement with an earlier report that transesterification reaction can occur at a different temperature range depending on catalyst and alcohol used [11].

The stoichiometric ratio for transesterification required 3-mole of methanol and a mole of oil to yield 3 mol of biodiesel and a mole of glycerol, which indicates that excess methanol, is required to drive the reaction toward the product, while enzyme load is also vital. This is because excess enzyme can make oils viscous, causing problem of mixing and demanding higher power consumption for adequate stirring [14–16]. Summary of methyl esters (FAME) present in both PO and PKO biodiesel interpreted from their respective gas chromatographs are described in Table 3. FAME present includes Hexadecanoic acid methyl ester, 9-Octadecenoic acid methyl ester, Dodecanoic acid methyl ester, 9, 12-Octadecadienoic acid methyl ester, 13-Octadecenoic acid methyl ester, 8-Octadecenoic acid methyl ester, Tetradecanioc acid methyl ester, 11-

Table 3 FAME composition of crude and transesterified palm oil (POBD) and palm kernel oil (PKOBD) from gas chromatography.

Methyl esters (FAME)	PO		PKO	
	CRUDE	POBD	CRUDE	PKOBD
Dodecanoate (Lauric acid)	0.09	18.32	–	–
Hexadecanoate (Palmitic acid)	–	4.54	1.32	13.7
Decanoate acid	–	–	–	–
9-Octadecanoate (Oleic)	–	6.52	–	15.61
Octadecanoate (Stearic acid)	–	1.48	–	2.34
9,12-Octadecanoic (Linoleic)	–	0.88	–	2.13
11-Octadecanoate acid	–	–	1.38	–
8-Octadecanoate	–	–	–	–
Tetradecanoate	–	–	–	–
13-Octadecanoate (Oleic acid)	–	–	–	–
Total	0.09	23.74	2.7	33.78

Table 4 Comparison of physic-chemical fuel properties of biodiesel from palm oil (POBD) and palm kernel biodiesel (PKOBD) with American and European fuel standards.

	STANDARDS		BIODIESELS	
	ASTM D6751	EN 14214	POBD	PKOBD
Pour point (°C)	–	(−15) − 10	6.7	17.7
Flash point (°C)	> 100–170	> 130	270	270
Density at 15 °C	880	860–900	813	851
Cloud (°C)	–	–	16.8	18
Kinematic viscosity (mm^2/s)	1.9–6.0	3.5–5.0	4.9	8

Octadecenoic acid methyl ester. Physico-chemical parameter of biodiesel produced from PO (POBD) and PKO (PKOBD) showed that POBD and PKOBD had pour point of 6.7 °C and 17.7 °C respectively, while both had a similar flash point of 270 °C (Table 4). The Kinematic viscosity of POBD (4.9 mm^2/s) was lower that determined in PKOBD (8.0 mm^2/s). Kinematic viscosity of PKOBD is in agreement with earlier report [13]. Also, both POBD and PKOBD had density of 813 kg/m^3 and 851 kg/m^3 respectively. High pour point of both biodiesels does not agree with ASTM 6751 and EN14214 standards respectively as they were higher than the accepted values. Similarly high flash point of both products is not acceptable with the ASTM standard, while it can be acceptable with European standard which sets no upper limit. However, Kinematic viscosity of POBD was within both standards while that of PKOBD was well above the standard Kinematic viscosity values. Conversely, densities of both POBD and PKOBD at 15 °C fell short of these standards. Better fuel properties of POBD compared with PKOBD could probably be due to the composition of fatty acid present. POBD has an average composition centered on two fatty acid- Palmitic (saturated) and Oleic (unsaturated), PKO is made up of majorly saturated fatty acid, while the unsaturated fatty acids are more responsible for the FAME properties [17]. Also, higher pour point, cloud point and flash point obtained for PO biodiesel compared to conventional petroleum based diesel were found to be consistent with earlier findings on such biodiesel fuel like alcohol esters of rapeseed, canola, beef tallow and soybean [18–20].

4. Conclusion

The present study showed that biodiesel with some fuel properties comparable with standards could be obtained from palm oil and palm kernel oil through trans-esterification by lipase enzyme, with palm oil showing better prospect. Further research is however needed to improve the quality of biodiesel produced through the use of processes such as further enzyme purification and also enzyme immobilization which could increase efficiency and cut cost.

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